ECOLOGY AND THERMAL INACTIVATION OF MICROBES IN AND ON INTERPLANETARY SPACE VEHICLE COMPONENTS

Twenty-ninth Quarterly Report of Progress

Research Project R-36-015-001

April 1, 1972 - June 30, 1972

Conducted by

Division of Microbiology - Cincinnati Research Laboratories
OS, Bureau of Foods
Food and Drug Administration

for the

National Aeronautics and Space Administration
Washington, D. C.

U. S. Department of Health, Education, and Welfare
Food and Drug Administration
1090 Tusculum Avenue
Cincinnati, Ohio 45226

October 1972

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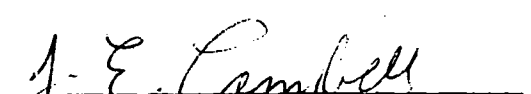

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Introduction

The sterilization parameters for the Viking lander of $D_{125°C}$ = 30 minutes and z = 21°C for the exposed bioburden were derived from the experimental findings of several laboratories conducting thermal inactivation studies on Bacillus subtilis var. niger by "dry heat." The moisture constraint--that the sterilizing gas shall be less than 25% relative humidity at standard conditions of 0°C and 760 mm Hg pressure--was added in recognition of the profound influence of water vapor on the time and temperature required for thermal inactivation of these spores.

The purpose of this report is to present data demonstrating that the application of the moisture parameter does not significantly change the $D_{125°C}$ and z values of 30 minutes and 21°C, respectively. Data are presented also to show the maximum influence that could be expected by decreasing the humidity to near zero % relative humidity at 105°, 113°, and 125°C.

EXPERIMENTAL

All experiments reported here were carried out in our conventional system. The spores were suspended in 95% ethyl alcohol, diluted in sterile double-distilled water, and dispensed with a repeating dispenser in 0.1-ml amounts in stainless steel cups to

give about 10^6 spores per cup. The cups were arranged on circular shelves and placed in 206 mm x 300 mm tin cans. Thirty cups were on each shelf and four shelves were used in each can for a total of 120 cups per can. The cans, lids, and contents were dried in a vacuum oven for 90 minutes at 45° to 50°C (at 1.5-inch Hg pressure absolute). To increase the drying rate, the oven was purged with dry nitrogen every 10 minutes for the first 70 minutes, and this was followed by five consecutive purges of nitrogen with a vacuum cycle between each purge. After drying, the cans, lids, and contents were removed from the oven and cooled to about 30°C in the equilibration hood. An appropriate amount of water or desiccant was placed in each can. The cans were sealed and removed from the equilibration hood. Heat treatments were applied as discussed below.

Viable spores were assayed by sonifying the cups in peptone water, and plating and counting on TGE agar. Prior to heat treatment, the seams on each can were soldered and wiped to preclude leakage of water vapor during heating cycle.

RESULTS

Thermal inactivation curves for B. subtilis var. niger at several temperatures under constant head space moisture $\simeq$ to 25% RH STP are presented in Figure 1. The D values range from about 26 minutes at 125°C to 18 hours at 95°C, with a z

value of about 20°C. The z value was calculated from data collected at 105°C and above.

Data are presented in Figure 2 for a similar study carried out under "dry" conditions through the addition of P_2O_5 to each can prior to heat treatment. We believe that the headspace moisture in these experiments did not exceed 0.001% RH at any temperature. D values ranged from 12 minutes at 125°C to 5 hours at 85°C, with a z value of approximately 30°C based on data collected from 100°C upward.

In Figures 3, 4, and 5 the appropriate data have been replotted so that the actual experimental findings can be compared to expected values derived from $D_{125°C}$ = 30 minutes and z = 21°C. It is seen that in each case (105°, 113°, and 125°C) the D value of the experimental results is equal to or less than the standard values. It is also seen that at all temperatures the D value can be reduced approximately by a factor of 3 by lowering the relative humidity of the system to near zero %.

SUMMARY

The addition of a parameter for maximum allowable water vapor in the sterilizing gas to be used in the thermal sterilization of the Viking lander has not significantly changed the D and z values for B. subtilis var. niger currently used for calculating the time and temperature of the thermal sterilization cycles.

INFLUENCE OF 1.3 ug (1.2125) H_2O/CM^3 OF HEADSPACE AIR ON THE INACTIVATION OF B. SUBTILIS VAR. NIGER AT SEVERAL TEMPERATURES.

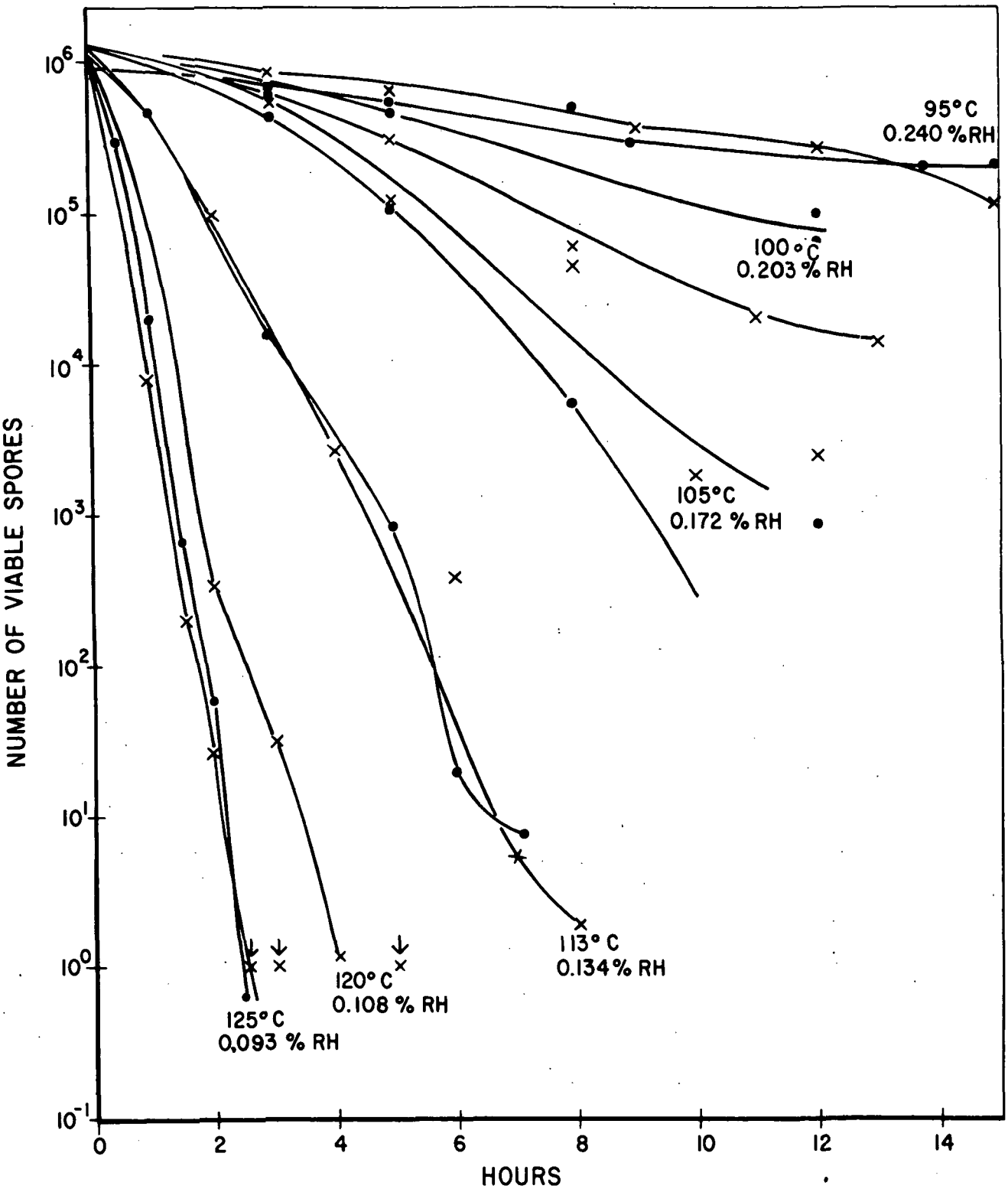

Fig. 1

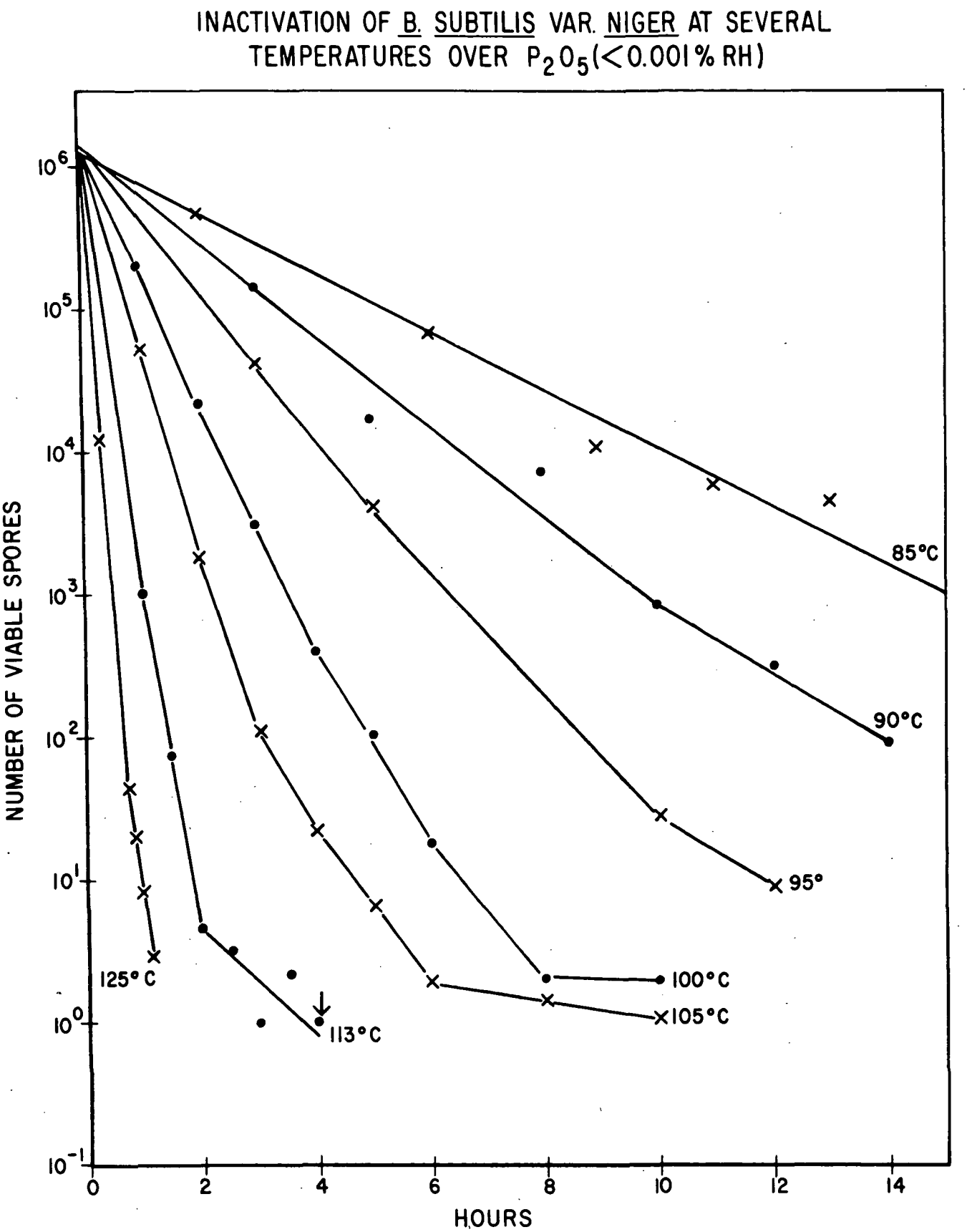
INACTIVATION OF B. SUBTILIS VAR. NIGER AT SEVERAL TEMPERATURES OVER P_2O_5 (<0.001% RH)
NUMBER OF VIABLE SPORES
10^6
10^5
10^4
10^3
10^2
10^1
10^0
10^{-1}
0
2
4
6
8
10
12
14
HOURS
85°C
90°C
95°
100°C
105°C
113°C
125° C

Fig. 2

INFLUENCE OF HUMIDITY ON THE INACTIVATION OF
B. SUBTILIS VAR. NIGER AT 105° C.

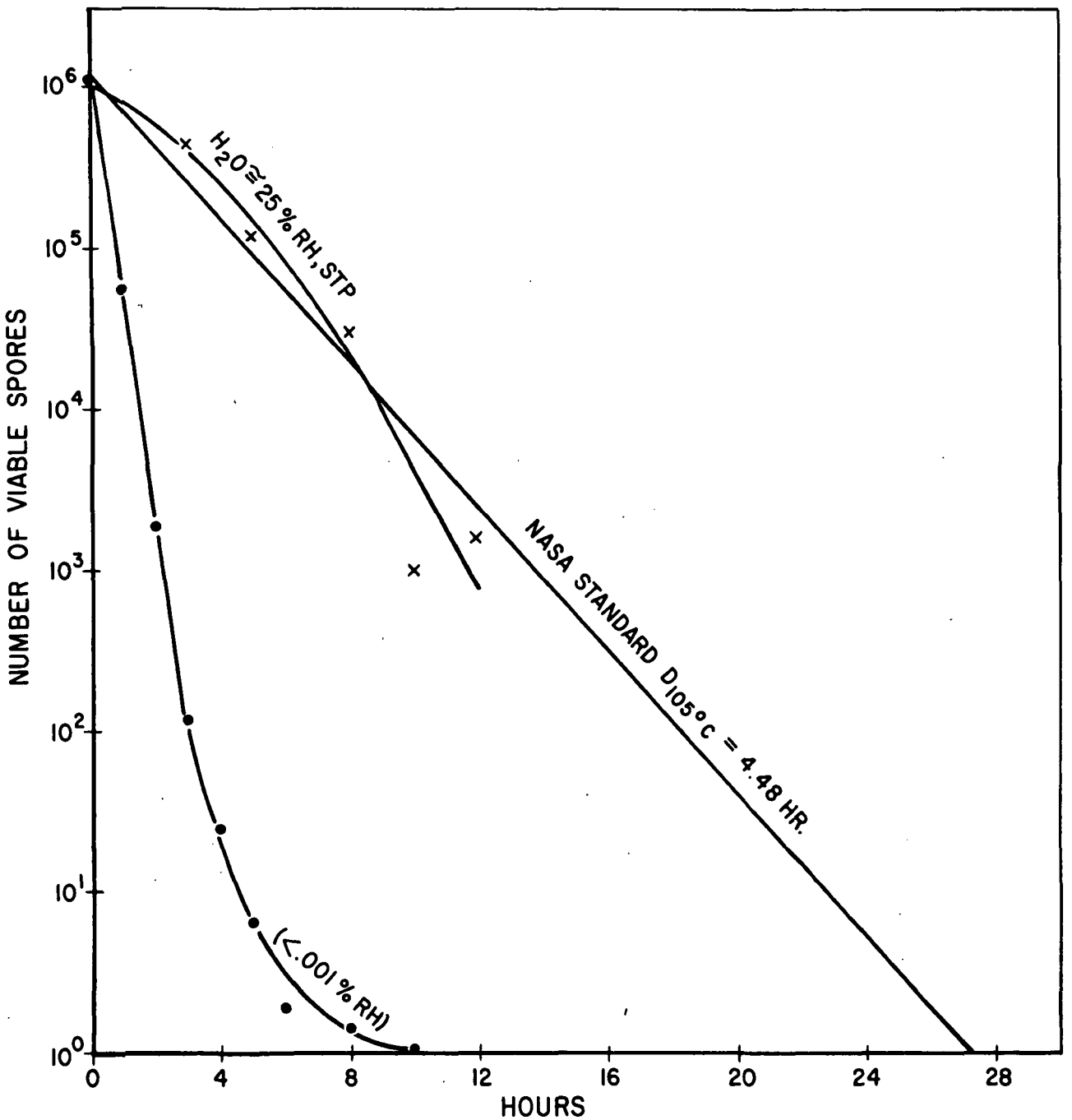

Fig. 3

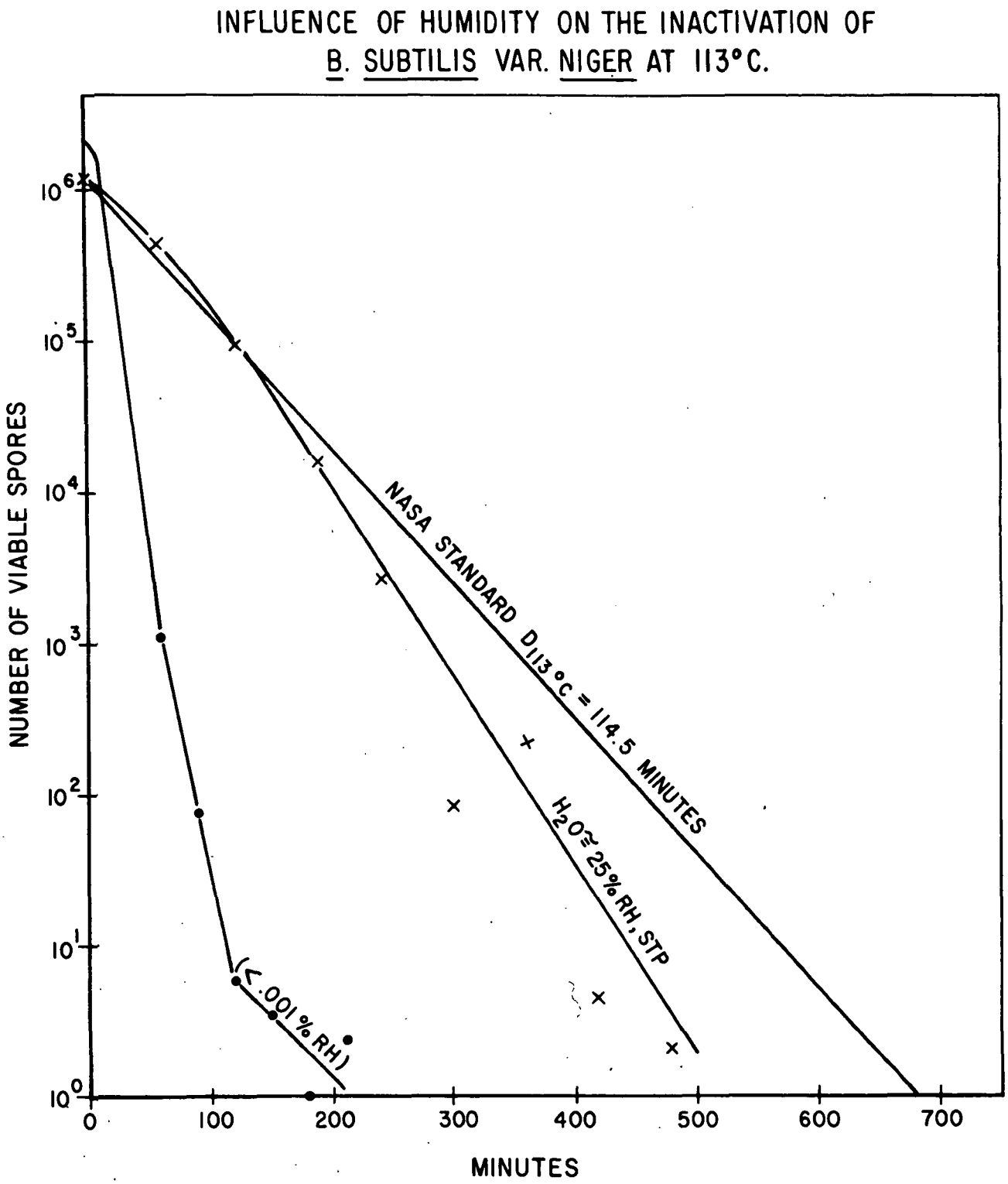
INFLUENCE OF HUMIDITY ON THE INACTIVATION OF
B. SUBTILIS VAR. NIGER AT 113°C.
NUMBER OF VIABLE SPORES
10^6
10^5
10^4
10^3
10^2
10^1
10^0
0
100
200
300
400
500
600
700
MINUTES
NASA STANDARD $D_{113°C}$ = 114.5 MINUTES
$H_2O \cong 25\%$ RH, STP
(< .001% RH)

Fig. 4

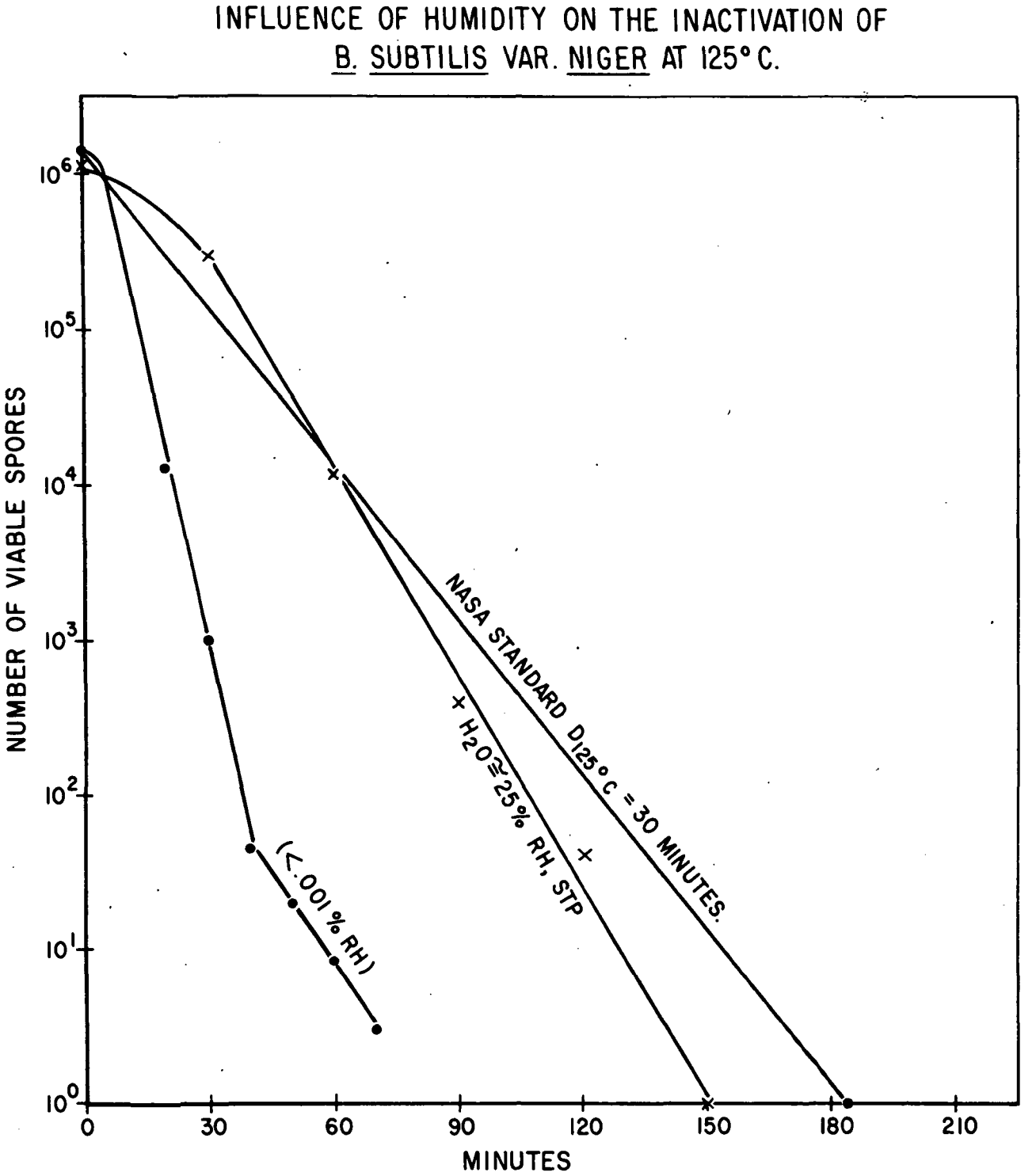
INFLUENCE OF HUMIDITY ON THE INACTIVATION OF
B. SUBTILIS VAR. NIGER AT 125° C.
NUMBER OF VIABLE SPORES
10^6
10^5
10^4
10^3
10^2
10^1
10^0
0
30
60
90
120
150
180
210
MINUTES
NASA STANDARD D125° C = 30 MINUTES.
+ $H_2O \geq 25\%$ RH, STP
(<.001% RH)

Fig. 5